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# THE DAILY ACTIVITY RHYTHMS OF THE HOLOTHURIANS IN THE CORAL REEF OF PALAO ISLANDS

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# THE DAILY ACTIVITY RHYTHMS OF THE HOLOTHURIANS IN THE CORAL REEF OF PALAO ISLANDS<sup>1)</sup>

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*With 7 Tables and 3 Text-figures*

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## I. Introductory Remarks

The author has visited Palao Islands twice, from January to July in 1937 and from July to September in 1940, for the study of holothurian ecology. The investigations were performed at the Palao Tropical Biological Station, which has been closed since World War II. The reports of the study were published in 1938, 1939, 1941 and 1955. Some interesting facts hitherto unpublished and an interpretation of these are reported in the present article. The author believes that these will add some contributions to the ecology and physiology of holothurians.

## II. Daily Rhythms of Feeding Activities

From the observations, carried out in 1937, on the behavior of holothurians in the coral reef of Palao Islands, the holothurians were classified into the following two groups according to their feeding habits (YAMANOUCI 1939, p. 634).

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1) Contributions from the Seto Marine Biological Laboratory, No. 284.

The A-group takes food uninterruptedly day and night, and always stays on the sandy flat (*Holothuria atra*, *H. edulis* and *H. flavomaculata*).

The B-group gives up feeding at least in one-third of the day (*Holothuria bivittata*, *H. vitiensis*, *H. scabra*, *H. lecanora* (JAEGER) var.,<sup>1)</sup> *Stichopus variegatus* and *St. chloronotus*). Among them *H. bivittata*, *H. vitiensis* and *H. scabra* are buried in the sand while fasting time from before dawn to the noon or up to the evening, whereas *Stichopus chloronotus* hides itself under corals instead of burrowing in the sand while fasting.

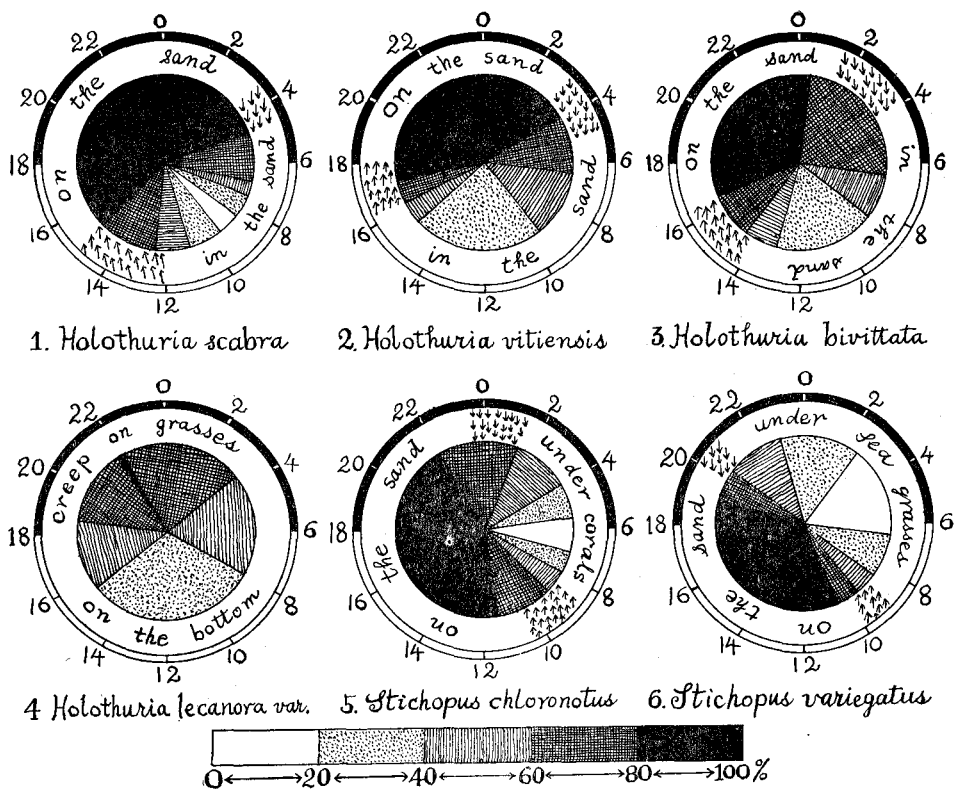


Fig. 1. The daily change in the amount of gut content, expressed in the percentage degree of fullness, in 6 holothurians inhabiting the coral reef of Palao Islands. The arrows indicate the times at which the holothurians had changed their activity grounds.

The behavior of *Stichopus variegatus* while fasting was finally made clear in the following investigation in 1940. During the fast, this species hides itself under sea grasses growing dottedly on the sandy flat (YAMANOUCHI 1941, p. 138).

The daily rhythms of feeding activity of these 6 species belonging to the B-group and its behavior are drawn schematically in Fig. 1. From this figures one sees that

1) Called by the native as "Akarumurm" (YAMANOUCHI 1939, p. 605).

4 species belonging to the Genus *Holothuria* begin actively to feed (↑) in the afternoon from the midday to the evening respectively following essentially the sun set (Fig. 1, 1-4), whereas two species belonging to the Genus *Stichopus* commence their feeding activity (↑) in accord with the increase of sun light nearly about 5-6 hours earlier than the *Holothuria* species (Fig. 1, 5-6).

In both groups the rhythm of external light seems to be surely the factor controlling the behavior rhythm of holothurians, and the apparent discrepancy of behavior from the external rhythm in each species should come from its intrinsic autonomous rhythm. The daily rhythms of behavior in holothurians above stated remind us of higher animals including the human being. Whether the fast period or the inactive state of six holothurian species represented in Fig. 1 corresponds to the sleeping hours of higher animals requires detailed analysis of holothurian behaviors. In the next two chapters this point will be specially considered.

In the lowest inactive state, the percentage gut contents of holothurians were as follows (given in brackets, cited from YAMANOUCI 1939, pp. 620-623): *Holothuria scabra* (14%), *H. vitiensis* (30%), *H. bivittata* (35%), *H. lecanora* var. (30%), *Stichopus variegatus* (5%) and *St. chloronotus* (10%). When and just after the animals moved into the sand or hid themselves under shelters, the feeding activity would not cease in all animals. Similar circumstances had been observed at the awaking state from resting. In some individuals, though not in all, feeding activity had been started considerably earlier than the actual time of coming out of the sand or from shelters (Table 1).

Table 1. Number of animals given according to the degree of fullness of their alimentary canal in different states of *Holothuria vitiensis*. 9-10/VIII, 1940.

State of animal	Just emerging from the sand	Having emerged from the sand	On the sand	Just burrowing into the sand	In the sand
Time	17.30	17-18	22	4	10
Degree of fullness					
100%	1	7	1	3	0
90	1	3	1	3	0
80	1	3	2	5	0
70	0	4	0	3	0
60	1	3	0	1	0
50	1	1	0	1	0
40	3	0	0	1	1
30	0	1	0	1	3
20	0	1	0	0	1
10	1	0	0	1	1
0	0	1	0	1	14
Total	9	24	4	20	20
Average content	57%	74%	88%	69%	8%

In *Holothuria vitiensis*, the amount of gut content of the burrowed animals was under 40% (0-40%, in average 8%) (Table 1), whereas at the exact moment of coming out of the sand or at the moment of burrowing into the sand, the amount of gut content fluctuated from 0 to 100% with wide individual variations. In *Holothuria bivittata* and *H. scabra* the circumstances were similar (Table 2). In higher animals food taking activity is entirely given up during sleep.

Table 2. Relation between the amount of gut content and the state of animals. The gut content is given in the percentage degree of fullness. The number in brackets denotes number of specimens observed.

State of animal	Just burrowing into the sand		In the sand		Just crawling out of sand		Within 1 hour after crawling out of sand		On the sand	
	Time	Average gut cont.	Time	Average gut cont.	Time	Average gut cont.	Time	Average gut cont.	Time	Average gut cont.
<i>Holothuria vitiensis</i>	4	69%(20)	10	8%(20)	17,30	57%(9)	15-18	74%(24)	22	88%(4)
<i>H. bivittata</i>	1,30	83%(10)	11	32%(14)	19	44%(13)	—	—	—	—
<i>H. scabra</i>	4,30	52%(18)	10-12	32%(24)	—	—	—	—	12-18	87%(54)

Interest had been specially concentrated seeking to know whether the burrowing holothurians would take sand or not. Fortunately by careful observation of the sandy flat of reef, it was found that the surface of the sandy flat was covered with sand of nearly whitish coloration, whereas a few cms. beneath the surface the sand was colored dark or nearly blackish as the result of bacterial fermentations. Therefore by dissecting the animal body the gut contents could be estimated from their colors, in what place they would have been taken, on the surface of sandy flat or in the interior of it. In Table 3, the color of ingested sand in *Holothuria bivittata* is tabulated. Materials dissected were brought from natural habitat at different times.

- A. at 1,30 o'clock, when they were just burrowing into the sand,
- B. at 11 o'clock, when they were burrowed in the sand,
- C. at 18-19 o'clock, within one hour after they had began to feed actively on the sandy flat.

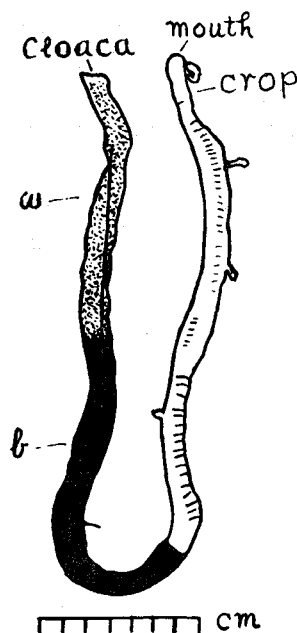


Fig. 2. The alimentary canal of *Holothuria bivittata* collected at 11 o'clock at which it was burrowed in the sand. The anterior half of canal was vacant. The posterior intestinal part was filled with two sorts (*b*, *w*) of sand. *b*: blackish or dark colored gut content taken by animal from the interior of bottom. *w*: the sand derived from the sandy surface. Copied from photograph.

Table 3. The color of ingested sand in the alimentary canal of *Holothuria bivittata*. Stomach and intestine are divided respectively into four parts from anterior to posterior. B, W or W-B denotes a blackish, whitish or intermediately colored gut content respectively, and — means vacant. Observation was carried out on 7/VIII in 1940.

A. Observed at 1,30 o'clock, when animals were just burrowing into the sand.

No.	Total gut content %	Blackish content %	Oesophagus and crop	Stomach				Intestine				Region of cloaca
1	0	0	—	—	—	—	—	—	—	—	—	—
2	100	30	B	B	B	W	W	W	W	W	W	W
3	100	20	B	—	W	W	W	W	W	W	W	W
4	50	10	B	—	W	W	W	—	—	—	W	—
5	80	10	B	W	W	W	W	W	W	W	—	—
6	100	10	B	W	W	W	W	W	W	W	W	W
7	100	10	B	W	W	W	W	W	W	W	W	W
8	100	30	B	B	B	W	W	W	W	W	W	W
9	100	20	B	B	W	W	W	W	W	W	W	W
10	100	20	B	B	W	W	W	W	W	W	W	W
Mean	83%	16%										

B. At 11 o'clock, when animals were found in the sand.

No.	Total %	Blackish %	Oes., crop	Stomach				Intestine				Cloaca
1	10	10	—	—	—	B	—	—	—	—	—	—
2	50	30	—	—	—	—	—	B	B	B	W	W
3	0	0	—	—	—	—	—	—	—	—	—	—
4	40	40	—	—	—	—	—	—	B	B	B	B
5	50	50	—	—	—	—	—	B	B	B	B	B
6	50	50	—	—	—	—	—	B	B	B	B	B
7	50	50	—	—	—	—	—	B	B	B	B	B
8	40	20	—	—	—	—	—	—	B	B	W	W
9	50	35	—	—	—	—	—	B	B	B	W	W
10	5	5	—	—	—	—	—	—	—	—	—	B
11	40	40	—	—	—	—	—	—	B	B	B	B
12	10	10	B	—	—	—	—	—	—	—	—	—
13	50	50	—	—	—	—	—	B	B	B	B	B
14	0	0	—	—	—	—	—	—	—	—	—	—
Mean	32%	28%										

C. At 18-19 o'clock, within 1 hour after they had begun to feed on the sandy flat.

No.	Total %	Blackish %	Oes., crop	Stomach				Intestine				Cloaca
1	100	10	W	W	W	W	W	W	W	W	W	B
2	10	10	—	—	—	—	—	—	—	B	—	—
3	30	30	—	—	—	—	—	—	—	B	B	B
4	80	40	W	W	W	W-B	—	—	B	—	—	B
5	80	50	W	W-B	W-B	W-B	W-B	B	B	—	—	B
6	10	5	W, B	—	—	—	—	—	—	—	—	B
7	40	40	B	—	—	—	—	—	B	—	B	B
8	10	10	—	—	—	B	—	—	—	—	—	—
9	60	50	W, B	—	—	—	—	B	B	B	B	W
10	30	15	W	W	B	—	—	—	—	—	B	—
11	40	20	W	W	—	—	—	—	—	—	B	B
12	80	50	W	—	W	W	B	—	B	B	B	—
13	10	10	B	—	—	—	—	—	—	—	B	—
Mean	44%	26%										

One example of the alimentary canal of dissected material is shown in Fig. 2.

The blackish ingested sand in the crop and in the anterior part of stomach indicated without doubt that holothurians swallowed sand from the interior part of the sandy flat during burrowing movement, what would have certainly facilitated their burrowing into the sand (Table 3, A). In 9 animals out of 14 specimens examined, the anterior half of the gut became vacant, while the posterior half was filled with dark colored contents and in 5 animals out of 14 almost the entire alimentary canal was vacant (Table 3, B). Judging from the amount and the site of the ingested sand in the gut, holothurians would have ingested sand to some extent not only during burrowing movement but also in the following early stage of resting in the sand. Then the animals would give up feeding totally, falling, so to speaking, into a "deep sleep." From C in Table 3, it is clear that some animals, though not all, would begin feeding hastily before coming out of the sand (No. 1, 4, 5 and 12). In *Holothuria bivittata* the time from ingestion to egestion of sand covers about 3-5 hours (YAMANOUCHI 1939, p. 612). This is what the characteristics of the gut contents exhibited in Table 3 would indicate as to what had actually happened in the feeding activities of holothurians within 3-5 hours before dissecting the animals.

### III. Respiratory Rhythms

By observing the cloacal pulsation during respiration in 8 holothurian species reared in the laboratory aquarium, some interesting results were obtained. In most animals, including man, breathing movement is accomplished by alternate inspiration and expiration of air or water. In holothurians the inspiratory pulsations intervening between two expirations or water spoutings number one or many. This number of inspirations was the thing of importance and of required detailed observation. In some species, for instance *Stichopus variegatus*, the number of inspirations numbered from 1 to 23 (Table 6). After repeated large numbered inspirations, a strong spouting of water followed, gushing out a large volume of water from cloaca. Also at the end of a long series of repeated inspirations, the amplitude of cloacal pulsation became much smaller than at the beginning. By counting the number of inspiratory pulsations between two expirations, it was revealed that there exist different types of respiration in connection with the activity rhythms of holothurians.

In Table 4 the frequency of the inspiratory numbers between two expirations, in three holothurian species in the different states of activity is summarized. In *Holothuria bivittata* and *H. vitiensis*, the 3 inspirations occupied about 50% of the total observed cases when the animals were in the active state of wandering on the sand. In the resting state of being burrowed in the sand, the 1 inspiration covered the range of 90% of the total observed cases. In *H. scabra* the 3 inspirations was in majority in four different activity states; however, at the moment of beginning the burrowing movement and throughout the resting state in the sand, this number of inspirations

Table 4. The frequency of the number of inspirations intervening between two expirations or between two spoutings in three holothurian species in different states.

Animal	<i>Holothuria bivittata</i>						<i>Holothuria vitiensis</i>						<i>Holothuria scabra</i>							
	On the sand		In the sand		Just emerging out of sand		On the sand		In the sand		Just emerging out of the sand		In the sand		Just burrowing into the sand		In the sand		Just emerging out of the sand	
	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%	cases	%
1	203	27.8	1383	88.4	13	21.3	44	14.8	620	91.6	13	25	4	1.4	—	—	1	0.3	—	—
2	24	3.3	10	0.3	2	3.3	1	0.3	—	—	—	—	1	0.3	—	—	—	—	1	1.0
3	385	52.7	157	10.0	44	72.2	161	54.1	59	8.4	16	30.9	181	59.2	40	93.0	275	92.4	34	35.9
4	13	1.8	3	0.2	—	—	3	1.0	—	—	1	1.9	3	0.1	—	—	—	—	—	—
5	69	9.2	10	0.7	1	1.6	55	18.5	—	—	10	19.2	89	29.1	1	2.3	14	4.7	32	33.7
6	10	1.4	1	0.0	—	—	1	0.3	—	—	—	—	1	0.3	—	—	—	—	—	—
7	18	2.5	1	0.0	1	1.6	17	5.7	—	—	10	19.2	23	7.5	2	4.7	6	2.0	22	23.2
8	1	0.15	—	—	—	—	1	0.3	—	—	—	—	—	—	—	—	—	—	1	1.0
9	4	0.7	—	—	—	—	10	3.3	—	—	1	1.9	1	0.3	—	—	1	0.3	3	3.2
10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	0.3	—	—
11	2	0.3	—	—	—	—	2	0.7	—	—	—	—	1	0.3	—	—	—	—	1	1.0
13	1	0.15	—	—	—	—	1	0.3	—	—	1	1.9	1	0.3	—	—	—	—	1	1.0
15	—	—	—	—	—	—	2	0.7	—	—	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—	—	—	1	0.3	—	—	—	—	—	—
Sum	730	100	1565	100	61	100	298	100	679	100	52	100	306	100	43	100	298	100	95	100
No. of animals	20		19		5		15		16		5		17		3		14		9	

covered over 90% of all observed cases. In three species the number of inspirations was more markedly distributed in a wider range when they were on the sand and wandering actively than when in the sand.

In another word, when being in the active cycle, the animals respired seemingly on their own accord, and when they were in the resting state being burrowed in the sand, they respired regularly or uniformly with as few inspiratory pulsations as possible. The regular and stereotyped respiration type in the resting state may be surely related with the necessity of holding their bodies firmly in the sand. This point will be discussed again in connection with the cycle of muscle tonus in the next chapter.

The uniform and stationary type of respiratory movement seems to come from nervous control. In *Holothuria scabra*, just burrowing animals respired in the same manner as in the sand (Table 4). This holothurian was able to exhibit a daily behavior rhythm following the environmental one even when the animal was entirely deprived of the visceral organs and body fluid (YAMANOUCI 1939, p. 625).



In holothurian respiration, the intake and expulsion of water are accomplished by the simultaneous cooperative movements of the respiratory trees and the bodily muscular system (CROZIER 1916). In swallowing water, the body wall was broadened and in expelling water out of the body through the cloaca, the circular muscles were in contraction to the effect that the body diminished in size. By the alternate broadening and contracting movements of the whole body in breathing movement, there occur random shaking movements of the medium surrounding the buried holothurian body. To maintain the burrowed state of the holothurian body for hours in the sand, a regular movement of the whole body, in another word, a regular breathing movement is required. In such a way the respiratory type of the 3 sand burrowing species of holothurians is specialized.

The range of distribution of inspiratory number, when the animals were on the sand, covered from 1 to 13 in *Holothuria bivittata*, 1 to 15 in *H. vitiensis* and 1 to 16 in *H. scabra* (Table 4). Whereas these numbers in the resting state in the sand numbered in majority 1 (88.4%) in *H. bivittata*, 1 (91.6%) in *H. vitiensis* and 3 (92.4%) in *H. scabra*. In addition to the interpretation made in the preceding sentence, the following explanation may be stated concerning the respiratory type. In holothurians the rôle of the epidermis is enormous in respiration as WINTERSTEIN<sup>1)</sup> (1908) pointed out that about 50% CO<sub>2</sub> is lost in the respiratory trees. In the sand epidermal respiration may be considerably reduced and hence the efficiency of normal respiration through the water-lung should be raised. A rapid respiration (i.e., small numbered inspirations) may be favorable in making gas exchange promptly than the deep breath (i.e., large numbered repeated inspirations) in the sand. The mean time interval of respiration was measured and listed in Table 5. By increasing the number

Table 5.<sup>2)</sup> Mean time interval of respirations of different types. 0 denotes inspiration, and | expiration or spouting. The number in brackets denotes the number of animals observed. Temperature of water 28.6–29.7°C.

Type of respiration	0		000		00000		0000000	
Animal	Mean in second	Number of resp.	Mean in sec.	No. of resp.	Mean in sec.	No. of resp.	Mean in sec.	No. of resp.
<i>Holothuria bivittata</i>	12.1	1369(17)	25.0	334(13)	38.7	24 (4)	46.1	4 (1)
<i>H. vitiensis</i>	11.3	663(14)	22.3	129 (9)	34.9	31 (4)	48.4	30 (4)
Mean	11.7		23.7		36.8		47.8	

of inspirations between two expirations, the respiration time interval became proportionately longer. The respiration accomplished by a large number of repeated inspirations would correspond to a "deep breath" in man. Such "deep breath" could not be observed when holothurians were buried in the sand in their resting state.

The holothurians are able to maintain the burrowing state by regulating the respiratory movement by (1) uniform and short respirations reducing the disturbance

1) Cited from CROZIER 1920.

2) Cited from YAMANOCHI 1941, p. 141.

of the medium surrounding the buried body to the lowest possible degree and by (2) relatively short respirations making the gas exchange as rapid as possible.

The frequency of the number of inspirations in 8 species of holothurians are summarized in Table 6. It is curious enough that the cases of odd number inspirations was extremely predominant over the even cases in all 8 species observed. Among 5403 (100%) cases, odd case counts 5259 (97%) and even case 144 (3%), and  $\text{Odd/Even} = 5259/144 = 43.5$ . Omitting the sum of inspiratory number 1 (2625) from the sum of odd cases (5259), then  $\text{Odd/Even} = 2634/144 = 19.2$ . And also omitting the sum of inspiratory numbers 1 and 3 (4217) from the sum of odd cases (5259), then  $\text{Odd/Even} = 1042/100 = 10.4$ . The reason for the predominancy of the odd cases over even ones is utterly inaccessible to the present author.

Table 6. Frequency of the number of inspirations intervening between two expirations in 8 species of holothurians.

Animal	No. of animals observ.	Number of inspiration between two spoutings																							Sum			Odd/Even
		1	2	3	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	23	Total	Odd	Even				
																						case	case	case				
<i>Holothuria bivittata</i>	20	1883	38	649	20	75	6	18	1	4	—	2	—	1	—	—	—	—	—	—	—	—	2694	2632	65	40.5		
<i>H. vitiensis</i>	16	733	2	247	3	65	1	24	1	11	—	2	—	2	1	1	—	—	—	—	—	—	1093	1085	8	135.6		
<i>H. scabra</i>	17	5	2	515	3	140	1	43	1	5	2	1	—	2	—	1	—	—	—	—	—	—	721	711	10	71.1		
<i>H. atra</i>	18	—	1	70	1	134	—	29	—	10	—	7	1	1	—	—	—	—	—	—	—	—	254	251	3	83.7		
<i>H. edulis</i>	14	—	—	13	—	47	3	39	1	14	1	7	—	5	2	—	1	—	—	—	—	—	133	128	5	25.6		
<i>H. lecanora</i> var.	5	—	1	8	—	13	—	7	—	3	—	—	—	—	—	—	—	—	—	—	—	—	80	79	1	79.0		
<i>H. sp.</i> <sup>1)</sup> (ngallau)	4	3	—	41	1	8	—	4	—	2	—	2	—	—	—	—	—	—	—	—	—	—	61	60	1	60.0		
<i>Stichopus variegatus</i>	15	1	—	111	164	30	107	5	20	2	6	1	4	4	—	3	1	2	1	1	1	364	313	51	6.1			
Sum	109	2625	44	1592	39	646	41	271	9	69	5	27	2	15	7	2	4	1	2	1	1	5403	5259	144	62.7			

In other 3 species (*Holothuria lecanora* var., *Stichopus variegatus* and *St. chloronotus*) which exhibited typical daily feeding rhythms (Fig. 1, 4-6) in the natural environment, it was extremely difficult in the laboratory to distinguish whether the animals were at rest or in the active state, as they were unable to burrow into the sand in the resting state, though in the natural environment the distinction of activity state was not difficult to make by observing a large number of animals on the sandy flat.

#### IV. Change of Muscle Tonus According to the State of Activity

The bodily shape and the mode of movement in holothurians are related with

1) Called by the Natives as "ngallau" or "ganrao" (YAMANOUCHI 1939, p. 628).

and adapted to their life in nature. In *Caudina chilensis* which except for the cloacal region is buried in the sand throughout life, the bodily shape is nearly circular in cross section (YAMANOUCI 1926, Fig. 1). Holothurians lying on the sea bottom generally have ventrally flattened and dorsally convexed bodily shape in cross section. In this state circular muscles and "muscle-like subepidermal elastic fibers (JORDAN 1914)" on the ventral side are in higher grade of tonus than those of the dorsal side (Fig. 3a). In the sand just the reversed relation is maintained (Fig. 3d).

The cross-sectioned bodily shapes on and in the sand remind us of an isosceles triangle. The base of triangle should be situated on the lower side for stability in the life of holothurians on the sandy flat. The reverse state, that is, the base of

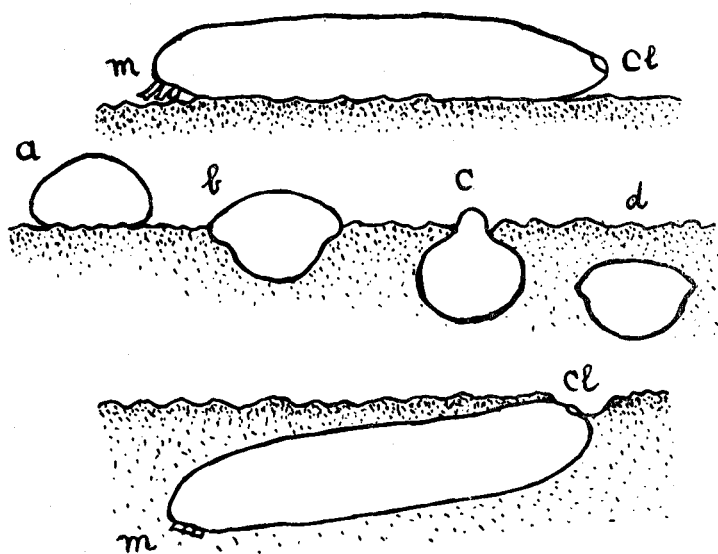


Fig. 3. Schematic drawings of *Holothuria bivittata* on and in the sandy bottom. *M*: mouth; *Cl*: cloaca. a, b, c and d denote the shape of the body in cross section when the animal burrows into the sand. Scale 1/5 natural size.

triangle being on the upper side, seems to be most stable when the animals are burrowed in the sand laying their bodies shallowly close to the bottom surface and holding that state for hours. To conquer the upheaving power coming from the disturbance of the surrounding medium by their breathing movements the only possible and reasonable shape of the body in cross section would be the one as drawn in d of Fig. 3.

The holothurians went into the sand "head" first aided by the anteriorly propagating peristaltic muscular waves along the body axis and at the same time by such muscular movements directed downward so that the lower side of the bodies wedged down into the sand (b in Fig. 3) and then it expanded to a circular shape in cross section forcing the sand aside (c in Fig. 3). After burrowing, the ventral side of the animal was stretched out downward continuously along the whole body

axis during the whole times of the "seemingly sleeping" state in the sand (Fig. 3). When the animals came out of the sand after the hours "seeming sleep," the process of movement was rather simple. The holothurians proceeded obliquely in an upward direction until they emerged on to the flat. In this instance the tonic relation of the dorsal and ventral circular muscles and muscle-like elastic fibers were reversed.

PEARSE (1908) reported the process of burrowing in *Thyone briareus* (LESSUEUR) which burrows into the sand, the middle portion of the body first, aided by the action of tube feet, the two ends of the body being turned upward. The process of the downward movement of the body during burrowing in *Thyone* are alike in the main features to those of the holothurians. According to him *Thyone* burrows completely into the sand within 2 to 4 hours. In the holothurians in Palao that time was within half an hour. The difference in the process of the burrowing movement and in its object between the two groups will explain the discrepancy of burrowing times.

### V. Reversal of Daily Activity Rhythms under Experimental Conditions

In a previous paper (YAMANOUCHI 1939, p. 624), the behavior of *Holothuria vitiensis* under artificial light in the laboratory has been reported. They had shown the same rhythmic activity of burrowing in and out of the sandy bottom as in the natural habitat, when illuminated from above by 100 watt electric light during the day time. When the aquarium was made totally dark by covers throughout the whole day, their activity rhythm was retained without change for several days, but after one week the daily rhythm became somewhat irregular. Uncovered, they had again exhibited their regular daily rhythm of activity.

In 1940, further experiments had been undertaken concerning the response of holothurians to varied light conditions. 7 specimens of *Holothuria vitiensis* were reared in the laboratory aquarium (80×50 cm, 17 cm sand depth and 50 cm water layer). Illumination of the aquarium was made by 100 watt electric lamp lighted 1 meter above the water level. For several days at the beginning natural light conditions were simulated, and then the light conditions were reversed, that is, during the day time from 6 to 18 o'clock the aquarium was made totally dark by covering it outside with thick black cloth and black paper, and during the night from 18 to 6 o'clock the next morning the aquarium was illuminated by electric light from above. The observation under reversed light conditions was continued for 10 days. The result is shown in Table 7. After three or four days under these unnatural lighting conditions, the activity rhythm of *H. vitiensis* had been completely reversed to accord with the new artificial light conditions. Reversing the light conditions back to the natural cycle, the normal rhythm of activity coming into and out of the bottom sand in accord with the daily cycle of light had been recovered after 2 or 3 days.

Table 7. Behavior of 7 specimens of *Holothuria vitiensis* in the aquarium under dark and light conditions. Number of animals is given, which wandered about on the bottom sand surface of the aquarium. 0 denotes all 7 animals being burrowed in the sand except for the cloacal region, therefore no individual could be found on the sand surface. 3 denotes 3 animals out of 7 being found on the sand surface and 4 hiding themselves in the bottom sand. Temperature of the sea water varied from 28.6° to 29.7°C. during 17 days (30/VIII-15/IX 1940).

Time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Day	← dark →							← light →										← dark →							
1	7	7					1			0			0			0	4			7	7				
	← light →							← dark →										← light →							
2		7					0		0		0	0			7	7			3		0				
3							0		0		0	0	1	1	1	7			3		3				
4							0				1		3			2	0	0			0				
5		4					3		5			3	1			0				0					
6							5			7	5		1			0			0						0
7	0							7	5		6		6		0	0	0		0						0
8	0						7		6		5				0			0							0
9	0							7	6		3		0			0						0			
10		0					2		2		3		0			0						0			
11							2		4		2		3			1						0		0	
	← dark →							← light →										← dark →							
12	0							3	1		1		0	0	0				0		0		0		1
13	1						5		0	0		0		0	0	1					5		3		
14	3	3				3		1	3		2		0			5			4						
15							1			1		1			2	1			6						5
16	5						0	0	0	1	1		0			5					7				
17							0		0																
Time	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

From this experiment, it was proved that the daily rhythm of activity in *Holothuria vitiensis* is dependent on the change of external light. The photosensitive area might be the cloacal region, for only this region was exposed to external light when the animals were burrowed in the sand. To prove this, the following procedure had been made: in the laboratory aquarium the cloacal region, continually pulsating for

respiration, had suddenly been illuminated by a strong light flux from a pocket electric lamp. *Holothuria bivittata*, *H. vitiensis*, *H. scabra* and *Stichopus variegatus* had reacted to the light beams with a momentary closure of cloaca. Moreover when several individuals of *H. vitiensis* and *H. scabra* were wandering about on the bottom of the darkened aquarium at midnight in the time of their normal active cycle, they were suddenly exposed to the light of a 100 watt electric lamp from above. Most of them had burrowed into the bottom sand after 30 minutes of continuous illumination.

## VI. General Considerations

To the group of holothurian feeding uninterruptedly day and night, are added two species inhabiting Japanese waters, *Caudina chilensis* (YAMANOUCI 1929, p. 75) and *Stichopus japonicus* (YAMANOUCI 1942, p. 344). Comparing the behaviors of 11 holothurian species observed by the author (3 belonging to A-group, 6 of B-group described in II and 2 of Japanese origin) the type of the daily activity rhythm is regarded to be the character common neither to Genus nor to Sub-genus of holothurians. It is a character specific to each species (YAMANOUCI 1942). But the species belonging to the same genus bear similar daily activity rhythms as pointed out in Chapter II.

The daily activity rhythms of holothurians were not precisely synchronized with the external light change. The change of sea water temperature was out of question in the tropical sea and the daily behavior rhythm was not correlated with the tidal rhythm (YAMANOUCI 1939, p. 623). The change of external light was the only external stimulus initiating the peculiar feeding rhythms in the 6 holothurian species previously mentioned. The deviations in behavior rhythms from the natural could be explained by the presence of an endogenous autonomous rhythm specific to each species. MORI (1945) has presented a good example on the relation between the behavior rhythm and the environmental one in the sea pen *Cavernularia obesa*. In this animal the pH change of the body fluid played an important rôle regulating the daily behavior rhythm induced by external light change. In holothurians alike metabolic change should be present, though this has not been investigated. But the case of holothurians was not so simple as that of the sea pen, as 7 specimens of *Holothuria scabra* whose entire visceral organs including body fluid being completely ejected from their cloaca by violent movement, were still able to exhibit normal daily rhythmic activity following the natural cycle of light change. The endogenous metabolic rhythm regulating the behavior should be also sought in such a case in the nervous system itself or in the surrounding medium in the body wall (YAMANOUCI 1939, p. 625).

Concerning the harmony of the part activity, that is, feeding activity (II), respiratory rhythm (III) and muscle tonus change (IV), in holding the "seeming sleeping" state in the sand, much has been said in the preceding chapters. But a little should

be added as to the origin of the sleeping phenomena of animals. There are some resemblances and differences between the sleep of higher animals and holothurians. The higher animals give up feeding during sleep. This is also true of holothurians, but in them feeding was not yet quite given up at the beginning of the burrowing state and it was begun already early before emerging on to the sandy flat. In another word in "shallow" seeming sleep, feeding was not yet given up in holothurians. In the higher animals nearly all muscles of the body, though not all, are relaxed during sleep, whereas in holothurians the dorsal tonic elements were in high tonic condition in contrast to the ventral. The feature of the change of respiratory movement in the awaking and resting states is in good agreement between the two.

The behavior pattern of holothurians in the "seeming sleeping" state in the sand should be ultimately attributed to their adaptation to the fact that holothurians breath with whole body movements in the sand. In the course of evolution, when the animals came to be provided with a central nervous system, especially the brain, sleep, in its true sense, is realized. In the evolutionary stage of possessing a brainless nervous system, the holothurians have exhibited the basic features characterizing sleeping phenomena.

## VII. Summary

1. A diagram representing the daily behavior rhythms of 6 holothurian species in the coral reef of Palao Islands was presented. In the species which burrowed in the sand during fast time (*Holothuria bivittata*, *H. vitiensis* and *H. scabra*), feeding was accomplished during the burrowing movement and in the early hour in the sand. It was begun before the time of emerging on to the sandy flat. In the middle period of resting in the sand, feeding was totally given up in these species. A method was found to determine in what place they would have taken sand on the surface or in the interior of the sandy flat.

2. In the sand the number of inspirations intervening between two expirations was maintained constant 1 or 3. In the active state feeding on the bottom sand, this number was variable, fluctuating from 1 to 16. Therefore the type of respiration in the sand should be regarded as stationary and stereotyped. By doing so, the holothurians held firmly their posture in the sand against the upheaving action of the surrounding medium and were able to make gas exchange as rapid as possible. The respiratory rhythm above stated seems to be under nervous control. The odd number of inspirations between two expirations was extremely predominant over the even one (Odd/Even=43,5).

3. The holothurians burrowed into the sand by anteriorly progressive peristaltic muscle waves and by the simultaneous downward directed alternate wedge-shaped stretching out and circularly expanding of the ventral side of the body. The tonus of the circular muscles and muscle-like elastic fibers was reversed alternately on the dorsal and ventral sides according to whether they were on or in the sand. The inverted

triangular bodily shape in cross section in the sand was favorable to maintain the buried state for hours.

4. The interesting facts which characterize the "seeming sleep" of holothurians should be attributed to their adaptation to burial in the sand during a limited time interval (8 to 13 hours) and their breathing with movements of the whole body.

5. Under inverted light conditions, illuminated during the night and made dark during the day, *Holothuria vitiensis* was found to be wandering on the bottom sand in the laboratory aquarium during the day time and buried in the sand during night in accord with the new lighting conditions. Their daily rhythm was thus proved to be dependent on the cycle of external light change.

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### Appendix. On the Pearl Fishes in the Palao Island

Among four species of pearl fish known in tropical seas, I have collected three species, that is, *Jordanicus gracilis* (BLEEKER), *Carapus parvipinnus* (KAUP) and *Carapus homei* (RICHARDSON) from several holothurians in the coral reef of the Palao Islands. On this I have already reported in 1938. In 1956 I was asked about pearl fish by Dr. LESTER R. ARONSON at the American Museum of Natural History. It would be by no means useless to describe the relation of the pearl fish to its symbiotic host holothurian. Moreover I have reported on holothurians containing poison in 1955 (YAMANOUCHI 1955). The venom of holothurian poison in concern is given in the last column of the next table.

Holothurian	Pearl fish found	Frequency of pearl fish found	Grade of the venom of holothurian poison
<i>Holothuria argus</i>	<i>Jordanicus gracilis</i> <i>Carapus parvipinnus</i>	ordinary	++
<i>H. bivittata</i>	sp. not recorded	ordinary	++
<i>H. flavo-maculata</i>	<i>Carapus homei</i>	rare	not tested
<i>H. lecanora</i> var.	not found	none	+++
<i>Stichopus variegatus</i>	<i>C. homei</i>	rare	++
<i>St. chloronotus</i>	<i>C. homei</i>	very abundant	++
<i>Thelenota ananas</i>	<i>C. parvipinnus</i>	ordinary	+++

The occurrence of pearl fish symbiotic in holothurians seems to be unrelated with the venom of holothurian poison, but related in the first place with the broadness of the holothurian cloaca, through which pearl fish can penetrate into the gut of its host.



For instance, in *Holothuria lecanora* (JAEGER) var. belonging to sub-genus *Actinopyga* which has small cloaca fringed with radially situated five hard horn-like epidermal processes, no single case of pearl fish symbiosis was found in the waters of Palao.

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